197-Symp
Phase Separation of Multi-Valent Signaling Proteins
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Cells are organized on length scales from Angstroms to microns. However, the mechanisms by which Angstrom-scale molecular properties are translated to micron-scale macroscopic properties are not well understood. We have shown that interactions between multivalent proteins and multivalent ligands can cause liquid-liquid demixing phase transitions, resulting in formation of micron-sized liquid droplets in aqueous solution and micron-sized puncta on membranes. These transitions appear to occur concomitantly with sol-gel transitions to form large, dynamic polymers within the droplets/puncta. I will discuss how such transitions may control the spatial organization and biochemical activity of actin regulatory signaling pathways, and contribute to formation of PML nuclear bodies in the mammalian nucleus. Our data suggest a general mechanism by which cells may achieve micron-scale organization based on interactions between multivalent macromolecules.

Platform: Voltage-gated Na Channels

198-Plat
Structure/Function Insights into Eukaryotic Channel Blocker Binding Sites in a Prokaryotic Sodium Channel
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Voltage-gated sodium channels are involved in electrical signalling in excitable tissues. They exhibit strong selectivity for sodium over other cations, thus enabling the cascade of events associated with action potentials. Mutations in these channels have been linked to a number of channelopathies such as epilepsy, cardiac arrhythmia, and chronic pain syndromes and therefore human sodium channels are targets for the development of local anaesthetics, antiepileptics, and other pore blocking drugs. Crucially, drugs which block eukaryotic channels have also been found to bind to and block bacterial sodium channels. Crystallographic, computational and electrophysiology experiments have been used to determine their functional effects and locations within the channel cavity. Their binding sites correspond closely to those previously predicted for these drugs in human sodium channels based on mutational studies, and may aid in the design of more specific and selective drugs.
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199-Plat
Intracellular Calcium Attenuates Persistent Current Conducted by Mutant Human Cardiac Sodium Channels in Long-QT Syndrome
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Mutation in the cardiac voltage-gated sodium channel $Na_V1.5$ can cause congenital long-QT syndrome type 3 (LQT3). Most $Na_V1.5$ mutations associated with LQT3 promote a mode of sodium channel gating in which some channels fail to inactivate, contributing to sustained or persistent sodium current ($I_{Na,P}$), which is directly responsible for delayed repolarization and prolongation of the QT interval. LQT3 patients have highest risk of arrhythmia during sleep or during periods of slow heart rate. During exercise (high heart rate), there is an increase in the steady-state intracellular free calcium (Ca) concentration. We hypothesized that higher levels of intracellular Ca may act in some way to lower arrhythmia risk in LQT3 subjects. We tested this idea by examining the effects of varying intracellular Ca concentrations on the level of $I_{Na,P}$ in cells expressing a typical LQT3 mutation, delKPQ. We found that elevated intracellular Ca concentration significantly reduced $I_{Na,P}$ conducted by delKPQ channels but not wild-type channels. $I_{Na,P}$ measured 200-ms after the peak transient current (expressed as % of peak current) for mutant channels was $2.6 \pm 0.4\%$ in low Ca and $0.6 \pm 0.1\%$ in high Ca ($p<0.001$). This attenuation of $I_{Na,P}$ in delKPQ expressing cells by Ca remained in the presence of KN-93 indicating it was not due to CaM kinase II activation ($2.2 \pm 0.2\%$ in low Ca and $0.9 \pm 0.3\%$ in high Ca; $p<0.001$). We conclude that intracellular Ca contributes to the regulation of $I_{Na,P}$ conducted by a LQT3 mutant and propose that, during excitation-contraction coupling, the increase of intracellular free Ca may contribute to suppression of mutant channel current and protect cells from delayed repolarization. These findings help explain the increased arrhythmia risk in LQT3 during slow heart rate.

200-Plat
Single-Particle Tracking Palm of Nav1.6 in Hippocampal Neurons Demonstrates Unique Subcellular Diffusion Landscapes
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The Nav1.6 isoform is one of the major Nav channels of the central nervous system. In addition to its AIS localization, this isoform is found at a lower density throughout the somatodendritic region of the neuron, where it is thought to contribute to the back-propagation of the action potential. Due to the low number of somatodendritic Nav channels, immunocytochemistry is not sensitive enough to detect these channels and instead electron microscopy is required to visualize them. Thus, the dynamics and diffusive behavior of these channels have never been previously observed in real time. To visualize this behavior, we have utilized a Nav1.6 construct tagged with the photoswitchable fluorophore, Dendra2 (Nav1.6-Dendra2). This construct was transfected into cultured rat hippocampal neurons and imaged via TIRF microscopy. We combined single-particle tracking with photoactivated localization microscopy (sptPALM) such that we tracked only a small subset of Nav1.6-Dendra2 molecules at any given time. A steady-state density of active fluorophores was maintained via a low-power activation laser and the trajectory of each molecule was determined using an automated detection and tracking algorithm. We typically obtained statistics of tens of thousands of trajectories in each cell with high spatial and temporal resolution. Then these trajectories were used to obtain a diffusivity map across the neuronal surface using a Bayesian inference scheme. Consistent with previous observations, this method showed that AIS Nav1.6 channels are stably anchored, presumably to AnkyrinG. In contrast, Nav1.6 channels in the somatodendritic region showed both diffusive behavior and periods of transient confinement within specific membrane regions, thus creating small membrane clusters. Interestingly, we found large, micron-size regions of membrane completely devoid of Nav1.6, which implies that the channel is physically excluded from these domains.

201-Plat
β4 Modulates $Na_V1.2$ Toxin Pharmacology
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The voltage-gated sodium (Na_v) channel signaling complex contains up to four distinct beta-subunits (β1-β4) that influence the functional properties of the pore-forming alpha-subunit. While investigating whether beta-subunits also influence ligand interactions, we found that β4 can drastically alter the response of the neuronal $rNa_v1.2a$ isoform to spider and scorpion toxins that target paddle motifs within Na_v channel voltage sensors. In conjunction with the β4 crystal structure, we utilized the altered sensitivity to a tarantula toxin as a tool to probe the interaction between $rNa_v1.2a$ and β4. As a result, we identified ^{58}Cys as an exposed residue that when mutated eliminates the influence of β4 on $rNa_v1.2a$ toxin pharmacology. In addition, we exploited β4-induced alterations in toxin sensitivity to probe the interaction between $rNa_v1.2a$ and a β4 mutant that mimics a β1 mutation implicated in epilepsy (C121W). We found that although this mutant still folds and traffics to the membrane, its interaction with the Na_v channel is lost. The principles emerging from this work: *1)* help explain tissue-dependent variations in Na_v channel pharmacology; *2)* enable the mechanistic interpretation of beta-subunit-related disorders; and *3)* provide new opportunities to design molecules capable of correcting aberrant beta-subunit behavior.

202-Plat
Distinct Voltage Sensor Gating of Cardiac Na_V Channels
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The channel-forming α subunit of voltage-gated Na^+ (Na_V) channels contains four domains (DI-DIV) each with six membrane-spanning segments (S1-S6). Voltage-clamp fluorometry (VCF) allows the tracking of the fourth charged segment (S4) in each domain with a site-directed fluorophore, which reports voltage-dependent changes in local protein conformation. We have created four novel channel constructs that enable us to study the voltage sensor

movements within each of the domains in the human cardiac voltage-gated Na^+ channel, $hNa_V1.5$, in conjunction with the cut-open oocyte technique. Our results with $hNa_V1.5$ show significant differences compared to previous VCF results from the rat skeletal muscle isoform ($rNa_V1.4$). Previously, the $rNa_V1.4$ DIII-S4 was shown to immobilize with kinetics that correlated with fast inactivation. In contrast, we show that $hNa_V1.5$ DIII-S4 rapidly returns to the down-state even when fast inactivation has not yet recovered. Based on this result, we hypothesize that the local anesthetic lidocaine, which was shown to alter the DIII-S4 voltage dependence in $rNa_V1.4$, also has a distinct interaction with $hNa_V1.5$. Supporting this hypothesis, we show no significant change in DIII-S4 voltage-dependence with the application of lidocaine to $hNa_V1.5$. Thus, previous lidocaine-$hNa_V1.5$ results that conflict with $rNa_V1.4$ VCF observations are now reconciled by our observation that the mechanism of interaction with the DIII-S4 differs between the two isoforms. In conclusion, despite high homology, significant functional differences exist between hNav1.5 and rNav1.4, in particular in the links between the DIII-S4, inactivation gating and lidocaine interaction.

203-Plat

Crystal Structure of the Navβ4 Extracellular Domain

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Voltage gated Na^+ channels (Nav) are present in all excitable cells and play a crucial role in the generation and propagation of action potentials. The eukaryotic channels are composed of a main α subunit together with one of four different auxiliary β subunits (β1-β4). These β subunits are largely extracellular, with a single transmembrane helix and a short cytoplasmic C-terminal tail. The extracellular domains of β subunits are thought to interact with exposed loops of the pore-forming α subunit and thusmodulate the functional propertiesof NaV channel.

Here we present the crystal structure of the extracellular domain of Navβ4 at 1.7Å resolution. The protein displays an immunoglobulin-like fold, with a conserved buried cystine bridge. A reactive cysteine (C58) is located at the surface and forms a likely interaction site with the α subunit. Recent unpublished functional data show that the C58A mutation causes a loss of Navβ4-mediated modulation. We mapped the positions of several disease-causing mutations identified in different Navβ subunits. One of these (C131W) affects the conserved buried cystine bridge, but surprisingly does not cause unfolding of the protein. Rather, a crystal structure of the mutant shows that it causes rearrangements in two distinct areas of the protein, including conformational changes in the loop containing the reactive cysteine.

204-Plat

Molecular Dynamics Studies of Ion Conduction in a Prokaryotic Channel

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Recently, the crystal structures of putative bacterial, voltage-gated sodium channels have been published, giving an atomic scale view of the NaChBac family of voltage-gated sodium channels. The selectivity filters of the open pore structure of NavMs (McCusker et al (2012) Nature Comm. 3, 1102) and the closed pore structure of NavAb (Payandeh et al (2011) Nature 475, 353) differ only on the order of 1-2 Å in diameter. Previously published studies aimed at predicting the mechanism of ion selectivity through free energy surfaces have not provided a consistent free energy surface. Electrophysiology studies suggest that sodium selectivity in the NaChBac family is slight when compared to the potassium selectivity in potassium-selective ion channels (Shaya et al (2011) PNAS 108, 12313). Multi-microsecond equilibrium simulations of NavAb by Chakrabarti et al. suggest that conformational movements of the glutamate side chains are correlated to sodium movement through the selectivity filter (PNAS (2013) 110, 11331).

We present microsecond timescale molecular dynamics simulations of ion conduction through a truncated model of the NavAb pore as a function of applied voltage, in solutions containing sodium, potassium, and both, and as a function of concentration. The model NavAb is constructed from the NavAb pore in which the S5 and S6 helices are truncated, creating an open pore, and embedded in a neon support. The S5 and S6 helices and the support are restrained, but the pore helices and selectivity filter are unrestrained. We show that conformational distribution of the glutamate side chains is voltage dependent. The dependence of selectivity on voltage, concentration, and cations present is demonstrated. Additionally, it is apparent that the rate of and predominant mechanism of conduction is not only dependent upon the voltage and cation but also upon the direction of current.

205-Plat

Phosphoproteomic Identification of CaMKII- and Heart Failure-Dependent Phosphorylation Sites on the Native Cardiac Nav1.5 Channel Protein

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Voltage-gated Na^+ (Nav) channels are key determinants of myocardial excitability and defects in Nav channel functioning or regulation, associated with inherited and acquired cardiac disease, increase the risk of life-threatening arrhythmias. In heart failure, the inactivation gating properties of Nav1.5 channels are altered, resulting in decreased channel availability and increased late Na^+ current. Although previous studies have suggested roles for CaMKII and CaMKII-dependent Nav1.5 phosphorylation sites, the global native phosphorylation pattern of Nav1.5 channels associated with these pathophysiological alterations is unknown. Mass spectrometric (MS)-based phosphoproteomic analyses were undertaken to identify in situ the native phosphorylation sites on the Nav1.5 protein purified from ventricles isolated from mice (CaMKIIdc-Tg) overexpressing CaMKIIdc in the heart. Quantitative analyses of Nav1.5 phosphopeptides allowed comparing the relative abundances of Nav1.5 phosphorylation sites in CaMKIIdc-Tg, versus wild-type, ventricles. A total of eighteen Nav1.5 phosphorylation sites were identified, seven of which are novel as compared with those reported in our previous LC-MS/MS analyses. Fourteen of these sites are located in the first loop, one in the second loop, one in the N-terminus and two in the C-terminus of Nav1.5. Interestingly, out of these eighteen phosphorylation sites, the C-terminal phosphoserine pS1938 is present in the CaMKIIdc-Tg IPs (n=3/4) and absent in the WT IPs (n=0/4), and pS1989 is 9-fold more represented ($p<0.05$, n=4 in each condition) in the CaMKIIdc-Tg, compared with the WT, IPs. Relative abundances of the other phosphopeptides, identifying the other phosphosites, are similar to the mean relative abundance found for all the other unmodified Nav1.5 peptides. These analyses (1) provide seven novel native cardiac Nav1.5 phosphorylation sites, and (2) identify two novel C-terminal Nav1.5 phosphorylation sites as potential critical determinants of the pathophysiological alterations of Nav1.5 channels in heart failure.

Platform: Molecular Dynamics I

206-Plat

Conformational Dynamics During GPCR - G Protein Coupling

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Upon activation by agonists, G-protein coupled receptors (GPCRs) in their active R* form transmit extracellular signals into the cell by catalysing GDP/GTP exchange in heterotrimeric G-proteins (Gαßγ, G). The GPCR rhodopsin activates Gt to transmit the light signal into retinal rod cells. After photon capture, the rhodopsin activity jumps by more than one billion fold implying both high fidelity and speed of active rhodopsin (RhR*)/Gt coupling. We employed all-atom molecular dynamics (MD) simulations to study the conformational diversity of membrane embedded rhodopsin and extend the static picture provided by the available crystal structures. An intrinsically unstructured cytoplasmic loop region connecting transmembrane helices 5 and 6 (ICL3) is identified. The MD data show how each protein state is split into several conformational substates. Only a single conformational substate with largely helical ICL3 is selectively stabilized by the Gtα C-terminus (GtαCT), as in the crystal structures. A mechanism for the fast and precise signal transfer from rhodopsin to Gt is proposed, which assumes a stepwise and mutual reduction of their conformational space. The analysis was extended to the interaction of GsαCT and GiαCT with activated β2-adrenoceptor (β2AR*) to address the issue of receptor promiscuity. We find that a short region of GsαCT stabilizes the open β2AR* conformation provided by the β2AR*/Gsαßγ crystal structure. In the crystal structures of RhR* with GtαCT, a close homologue of GiαCT, RhR* exhibits a more closed active conformation. With the slim GiαCT peptide we find that β2AR* adopts a similarly closed conformation as RhR* with GtαCT. This analysis elucidates how G-protein coupling specificity relies on the fold and primary structure of the Gα C-terminus.

Scheerer et al. (2008), Crystal structure of opsin in its G-protein-interacting conformation, NATURE
Elgeti et al. (2013) Precision vs Flexibility in GPCR signaling, JACS